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MIPR NUMBER: 95MM5537

TITLE: Protective Immunity to Hepatitis B and Streptococcus Pneumoniae in Active Duty Women Versus Men: Prevalence and Responses to Preventive Immunization

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REPORT DATE: April 1996

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1996	Final (1 Dec 94 - 31 Dec 95)

4. TITLE AND SUBTITLE Protective Immunity to Hepatitis B and Streptococcus Pneumoniae in Active Duty Women Versus Men: Prevalence and Responses to Preventive Immunization

5. FUNDING NUMBERS
95MM5537

6. AUTHOR(S)
COL Renata J.M. Engler

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Walter Reed Army Medical Center
Washington, DC 20307-5001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, 6 Jul 96). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200)

Specific antibodies to hepatitis B (HB) surface antibody (HBsAb) and 12 serotypes of *S. Pneumoniae* (1,3,4,6B,7F,8,9N,12F,14,18C,19A/F,23F) in a population of healthy active duty women and men (previously immunized for HB) were assayed in order to determine the need for further immunization with hepatitis B or pneumococcal polysaccharide vaccines. Participants identified with low levels (< 50 mIU/ml) of HBsAb were offered booster immunization by either an intradermal (ID 2 mcg) or intramuscular (IM 20 mcg) route and response was measured 4 weeks later. In 211 subjects (109 females; 102 males), the prevalence of HBsAb levels below 20 mIU/ml was over 15% with no significant gender differences. By age group, subjects over 35 years of age tended to have lower levels of HBsAb. Booster HB vaccine responses in 35 subjects (17 IM vs 18 ID) were not significantly different. Booster HBsAb levels were significantly higher in women than men ($P<0.05$). The prevalence of non-protective antibody levels (</= 200 ngAbN/ml) to pneumococcal serotypes 6, 7, 8, 9 and 14 was greater than 20%, regardless of age or gender. **Conclusions**: Loss of protective immunity to hepatitis B occurs in active duty men and women; a cost effective dosing regimen (2 mcg ID) can boost waning HBsAb levels. Active duty personnel should be considered for pneumococcal polysaccharide vaccination if placed in high risk environments, particularly for select serotypes where natural immunity is poor.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Defense Women's Health Research Program Hepatitis B, Pneumococcal, immunization, vaccination, immunity	39
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

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Renate JM Engler 4 July 96

PI - Signature Date

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Abstract

Specific antibodies to hepatitis B (HB surface antibody or HBsAb) and 12 serotypes of *S. Pneumoniae* (1, 3, 4, 6B, 7F, 8, 9N, 12F, 14, 18C, 19A/F, and 23F) in a population of healthy active duty women and men (previously immunized for HB) were assayed in order to determine the need for further immunization with hepatitis B or pneumococcal polysaccharide vaccines. **Methods**: Levels of HBsAb and serotype specific IgG to pathogenic pneumococcal bacteria were measured by enzyme immunoassays. Participants identified with low levels (< 50 mIU/ml) of HBsAb were offered booster immunization by either an intradermal (ID 2 mcg) or intramuscular (IM 20 mcg) route and response was measured 3-4 weeks later. **Results**: Two hundred and eleven subjects (109 females and 102 males) completed phase one of the study. The prevalence of HBsAb levels below 20 mIU/ml was over 15% with no significant gender differences. By age group, subjects over 35 years of age tended to have lower levels of HBsAb. Booster HB vaccine responses in 35 subjects (17 IM vs 18 ID) were not significantly different but trended higher in the ID group; booster HBsAb levels were significantly higher in women than men ($P<0.05$). The prevalence of non-protective antibody levels (</= 200 ng Ab N/ml) to pneumococcal serotypes 6, 7, 8, 9 and 14 was greater than 20% in all subjects, regardless of age or gender. **Conclusions**: HB vaccination response as measured by quantitative HBsAb after the primary series of immunization will identify those individuals who require a booster dose of vaccine. A cost effective dosing regimen (2 mcg ID) can effectively boost waning HBsAb levels. Active duty personnel should be considered for pneumococcal polysaccharide vaccination if placed in high risk environments, particularly for select serotypes.

Introduction

Active immunization of military populations is a time-honored preventive medicine intervention to minimize the health risks of soldiers during travel or job related activity (such as health care delivery or during stressful maneuvers known to increase certain disease risks). The efficacy of vaccination programs in terms of reductions in both morbidity and mortality of populations has been well established.[1-4] The utilization of certain vaccines in select high risk populations (e.g., hepatitis B subunit and pneumococcal polysaccharide immunogens) is based on the prevalence of lack of protection and the risk of disease.

Hepatitis B surface antigen (HBsAg) vaccines are currently recommended for active duty personnel with increased risks to contract this serious, chronic viral infection (e.g., health care workers, overseas deployment to high risk endemic areas). Vaccine is administered in a primary series at the present time but there are no recommendations regarding booster vaccination. Some reports in the literature have suggested that the route and schedule (timing of injections) are more important for optimizing protective antibody response than dose alone.[5] Others have suggested that protective antibody titers may be lost 3 to 5 years after primary immunization.[6] Women with hepatitis B infection present an increased risk for complications of pregnancy; hepatitis B infections transmitted from mother to infant result in a high risk of chronic active hepatitis.[7] There is currently no clear data available regarding differences in duration of vaccine response between men and women nor optimum and most cost effective strategies for supplemental or booster vaccine dosing.

It was the primary purpose of this study to determine the prevalence of loss of protective levels of hepatitis B surface antibody in a target population with a prior history of

hepatitis B immunization and to determine if gender differences were significant. In those subjects identified with non-protective (</= 10 mIU/ml) or low level HBsAb (less than or equal to 50 mIU/ml), response to booster vaccination by one of two different strategies (2 mcg intradermally versus 20 mcg intramuscularly) was compared by route and gender.

Pneumococcal disease can produce life-threatening infections including pneumonia, sepsis and meningitis. As one of the polysaccharide encapsulated bacterial pathogens, this group of bacteria generates a humoral immune response by a different pathway than standard protein immunogens. A vaccine is currently available, including 23 bacterial serotypes known to be pathogenic (reflecting over 90% of disease associated with *S. pneumoniae*), and has been shown to be effective in select groups of patients such as the elderly and chronically ill.[1,2] Significant natural immunity has been observed in young adult males, but natural immunity in women at different ages remains less well defined.[8,9] Among elderly adults, women have lower antibody levels to pneumococcal serotypes than men but they respond well to the vaccine.[9] The currently licensed pneumococcal vaccine is not included in the standard vaccinations for active duty military. The prevalence of immunity to pathogenic *Streptococcus pneumoniae* in adult populations is currently not well defined. A study of military recruits demonstrated anticapsular antibody to only 15% of common pneumococcal serotypes whereas working men had IgG to 33% of the serotypes associated with significant disease. Protective immunity increased to over 78% after vaccination with the 23 valent pneumococcal vaccine. This study suggested that subsets of young healthy adults should be considered for vaccination.[8] Since pneumonia among military recruits is caused by *S. Pneumoniae* in up to 30% of cases and other serious infections may occur (meningitis, sepsis), data on the prevalence of natural immunity in

active duty women and men would be helpful for future planning of more effective immunization strategies for the prevention of disease.

Active duty men and women, particularly in the health care professions, have an occupational increased risk for infection and secondary morbidity. The 23 valent pneumococcal polysaccharide vaccine has not been formally recommended for healthy adults because of the presumption of natural immunity due to environmental exposure. It was the secondary purpose of this study to determine the prevalence of protective immunity to 12 different pathogenic pneumococcal polysaccharide serotypes in active duty women (in different age groups) compared to active duty men.

Methods and Materials

This protocol was approved by the WRAMC human use committee and appropriate institutional review board. Active duty men and women (with a history of hepatitis B vaccination for occupational risk indications) were recruited from the Walter Reed Army Medical Center Immunization Clinic and the National Capital Region. An initial health related questionnaire was completed by each participant. Exclusion criteria for protocol participation included the following: malignancy, pregnancy, immunosuppressive therapy including corticosteroids, autoimmune diseases such as systemic lupus erythematosus, prior hepatitis B infection or any active systemic infectious disease. Each study subject's immunization history was reviewed regarding prior hepatitis B and pneumococcal polysaccharide vaccination. Patients with prior immunization with pneumococcal polysaccharides were excluded from the study. Baseline studies were performed in the institutional diagnostic immunology laboratory in order to establish the presence of normal humoral and cellular immunity (quantitative

immunoglobulins, IgG subclasses, specific antibody titers to tetanus, diphtheria, measles, mumps, rubella and hemophilus influenzae type b; complete blood count with absolute lymphocyte count; delayed hypersensitivity skin testing with the control recall antigens tetanus, candida and mumps). Normal humoral immunity was defined as protective specific antibody titers to at least 3 out of 6 recall antigens screened with normal IgG subclass levels. Normal cellular immunity was defined as normal range lymphocyte counts and delayed hypersensitivity skin test responses to at least 1 out of 3 recall antigens. Serum was stored on each subject at minus 70° centigrade for later assay in batches for quantitative hepatitis B surface antibody (HBsAb) and core antibody (HBcAb) as well as IgG specific for pneumococcal polysaccharide serotypes 1, 3, 4, 6B (26), 7F (51), 8, 9N (9), 12F (12), 14, 18C (56), 19A/F (19), and 23F (23) (Danish classification system used worldwide with U.S. system notation in parentheses if different).

Those subjects identified with hepatitis B surface antibody levels equal to or less than 50 mIU/ml were offered enrollment in a second phase of the study: randomization to receive a single booster of recombinant hepatitis B surface antigen vaccine by one of 2 schedules: 2 micrograms intradermally or 20 micrograms intramuscularly using the vaccine **Engerix B™**. Serum was collected and stored 3-4 weeks after the booster vaccine dose in order to be assayed with the pre-vaccine serum in a paired fashion. Protocol participants were advised that the current standard for "protective level" of HBsAb is 10 mIU/ml and that booster immunization for levels between 10 and 50 mIU/ml was elective for the purpose of the protocol but would provide long term immunity if levels were raised over 100 mIU/ml.

Quantitative serum hepatitis B surface antibody titers were detected using a commercially available kit (AUSAB® Quantitation Panel, Abbot Diagnostics Division, Abbott Park, North Chicago, IL 60064, 708-937-6100) according to the manufacturer's recommendations, in a single laboratory. HBsAb levels were converted to mIU/ml using a standard serum. Vaccinees were considered seroprotected when the HbsAb levels were greater than 19 mIU/ml although published reports suggest that levels below 10 mIU/ml are associated with the greatest risk of natural infection.[10] Levels below 20 mIU/ml roughly correlated with a negative qualitative HBsAb screening in our institutional laboratory.

Semiquantitative (positive/negative) Hepatitis B core antibody levels were detected using a commercially available kit (Hepatitis B Core Antigen (Recombinant) Corzyme®, Abbot Laboratories, Diagnostics Division, Abbott Park, North Chicago, IL 60064, 708-937-6100) according to the manufacturer's recommendations, in a single laboratory. This is an enzyme immunoassay for the qualitative determination of total antibody to hepatitis B virus core antigen in serum or plasma. Subjects having a positive screening HBcAb underwent additional testing for liver function tests, HBsAg, HBeAg & Ab, and PCR analysis for the presence of hepatitis B virus DNA in the serum. In the absence of any other markers of hepatitis B infection, subjects were not excluded from the study in view of the high rate of false positive HBcAb measurements.[11]

Statistical analyses were carried out using a statistical computer software package, (SPSS 6.1 for Windows, SPSS Inc., 444 N. Michigan Ave, Chicago, IL 60611, 312-329-2400; Fax 312-329-3668). Percentages of low level, non-protective HBsAb in each group were compared using Chi square or Fisher exact test. Geometric mean titers were compared after

logarithmic transformation using variance analysis or Wilcoxon's test when normality was not met.

Pneumococcal polysaccharide specific IgG levels to individual serotypes are reported in nanograms of antibody nitrogen per mililliter (ng Ab N/ml). Sera were assayed in batches using a standardized immunoassay system of a single commercial laboratory. (Specialty Laboratories, 2211 Michigan Avenue, Santa Monica, CA 90404-3900, Fax, 310-828-6634) Geometric means for each serotype as a whole and by gender and age grouping were calculated with the standard error of the mean (SEM). Comparisons were made using non-parametric analysis (Kruskall-Wallis) and analysis of variance (ANOVA). Correlations to age were also performed.

Results

Two hundred and twelve subjects (212) were enrolled in the study between December 1994 and December 1995 with 211 completing the initial phase of the study. The demographics of this group included 109 women and 102 men between the ages of 19 and 55 years. **Table 1** details additional demographics by age grouping and gender. There was no significant difference in mean ages by gender between each of three groupings: group 1 (GRP 1, 18 to 34 years), group 2 (GRP 2, 35 to 44 years), and group 3 (GRP 3, 44 to 55 years). The study population as a whole and by age grouping or gender grouping was defined as immunologically normal as described in materials and methods (data not shown). A single subject experienced a transient reaction to the delayed hypersensitivity skin testing with mumps antigen. Aside from a large local reaction, a generalized maculopapular rash persisting for one

week and requiring a short course of oral corticosteroids was documented. There were no adverse long term sequellae.

Table 2 summarizes the geometric mean HBsAb levels for the group as a whole and by gender. There was no significant difference between groups. After the initial enrollment of personnel with a history of a primary series of hepatitis B vaccine, 30 (14.2%) subjects presented with additional records demonstrating the fact that only 1 or 2 hepatitis B vaccine doses had been given rather than the primary series as initially recorded by history. Although the participants insisted they had received all 3 doses, the fact that all shot records were retrieved and only 2 doses were documented, resulted in exclusion of this population from further analysis for HBsAb levels. The lower half of **Table 2** summarizes the geometric means for the subgroup with documentation of at least 3 doses of vaccine. Again, there was no difference between groups. It is noteworthy however, that 18% of this immunized group had HBsAb levels below 20 mIU/ml which correlates to a negative screening HBsAb (positive/negative) in our laboratory. Absolute levels of HBsAb did not show significant correlation with years from last dose and some of the highest HBsAb levels were found in participants whose last vaccine dose had been more than 7 years earlier. Not shown in tabular form, the geometric mean HBsAb levels were not significantly different between groups categorized by years since last dose of vaccine (1-3, 3-5, 5-7 and over 7 years).

Table 3 summarizes the geometric mean levels of HBsAb by age grouping. For the age groups as a whole (male and female), there was a significant difference in the over 34 years of age groups compared to the younger population (18 to 34 years). However, there was no significant difference between groups for the number of subjects with HBsAb levels below 20

mIU/ml, ranging from 14 to 21 percent. When analyzed by gender grouping, this age grouping difference was reflected in males but not in females.

Hepatitis B core antibody screening was positive in 5 of the protocol subjects. Subsequent serologic screening for liver function, hepatitis B surface antigen, e antigen, e antibody and PCR for hepatitis B DNA were negative. Two of the 5 subjects converted to negative HBcAb on repeat screening. Consistent with the most recent reports of the high frequency of false positive HBcAb screens, these data supported that the subjects had not had a true natural infection and they were not excluded from the study.[10] None of these subjects were included in the groups receiving additional vaccine.

Table 4 summarizes the geometric mean levels of HBsAb in those subjects enrolled for the second phase of the hepatitis B phase of the study. One group received the booster dose by the intramuscular route (IM) at 20 mcg and the other by the intradermal route (ID) at 2 mcg. There is no significant difference between the ID and IM groups as a whole. **Figure 1** illustrates individual graphs for each of the vaccine recipients by route of administration. Each group had a similar number of non-responders who subsequently were referred to the vaccine clinic for evaluation and further immunization. None of the ID vaccinated non-responders raised an antibody response to a single IM dose. Two of the IM non-responders did respond to additional booster doses by the ID route. **Table 4** also displays the data by gender grouping. Noteworthy is the significantly higher geometric means for females compared to males for both the IM (2175 versus 113 mIU/ml) and ID (3393 versus 210 mIU/ml) routes of vaccination. However, the male grouping is smaller and includes a disproportionate number of non-responders and might reflect some sampling error due to the size of the study population. **Table 5** combines the IM and ID groups since the differences in response by route were not

statistically significant. Once again, the mean HBsAb level in the female group, independent of vaccine strategy, was significant higher (2716 versus 158 mIU/ml).

Geometric mean levels of pneumococcal polysaccharide specific antibody (in nanograms antibody nitrogen per ml or ng Ab N/ml) for serotypes 1, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19A/F, and 23F are summarized in **Table 6** for the group as a whole and by gender. Age grouping comparisons, also shown in **Table 6**, are not displayed by gender since no significant differences within these age groupings were found. **Table 7** summarizes the percent (%) of subjects in the population as a whole and by age grouping who had non-protective levels of antibody (</= 200 ng Ab N/ml) by serotype. Taking the population as a whole, more than 20% of subjects were non-protected for serotypes 6A, 7F, 8, 9N, and 14. When evaluated by age grouping, other serotypes demonstrated significant percents of the population who lacked serologic immunity. None of the study subjects had received a pneumococcal vaccine previously. It should be noted that serum antibody concentrations between 200 and 300 ng AB N/ml are considered the threshold of protection against pneumococcal infection. When 300 ng AB N/ml is used as the cut off, the percentages of protocol subjects with "low level" antibody increases to over 50% in many of the subgroups listed in the 20-30% range in **Table 7**.

Discussion

Hepatitis B Virus Specific Immunity

Optimizing the protective immunity of active duty personnel by the appropriate use of available immunogens has been the cornerstone of medical readiness, occupational health and preventive medicine for the troops. Subpopulations of active duty personnel, such as health

care providers and deploying troops to developing countries have a particular need for hepatitis B protective immunity. Currently these high risk personnel are supposed to receive a primary series of hepatitis B vaccination. Our study demonstrates that more than 10% (30 out of 211 enrolled) of the population sample had not completed the series despite the formal recommendation of a primary series and access to a major medical center with a walk-in service for immunizations. Up front, this illustrates the need for better documentation, via an automated database system, of immunizations received and a method for notifying those individuals with inadequate follow-up immunization.

In the pediatric population, the goals of immunization are primarily herd immunity with individual protection a secondary goal. In high risk populations however, optimizing individual immunity assumes greater importance. In our study, 18% of subjects with at least a primary series of immunization failed to have clearly protective serologic immunity as measured by HBsAb > 20 mIU/ml. Since this observation was independent of time since last dose of vaccine, the question is raised whether or not the high risk populations should have routine serologic screening at some interval and then receive booster dosing versus booster dosing without serologic screening. Our study does not provide useful information as to the ideal timing for a booster since levels did not correlate to years from initial vaccination. Rather the data supports previous observations that high responders maintain high levels over longer periods of time (> 10 years) and low responders may require more frequent boosters.[6,12-16]

Krugman and his co-authors reported on 54 seropositive health care workers (13% low responders at 1 year) with 5 to 7 year follow-up demonstrating loss of protection (HBsAb < 10 mIU/ml) in 48%. Jilg recommended that if initial vaccine response was below 100 mIU/ml that a booster be given within 1 year and that those above 100 mIU/ml should receive a booster at 5

to 7 years.[16] Most recently, Tilzey and his co-workers recommended that subjects between 10 and 500 mIU/ml should be boosted immediately and those between 500 and 4000 should receive a booster at 5 years; those over 4000 potentially do not need a booster for at least 10 years if not longer.[17] This approach minimizes the number of antibody tests after initial response is documented, saving occupational health personnel the problem of long term follow-up and repeated semi-quantitative testing (positive/negative only around 10-20 mIU/ml). Not addressed was the issue of waning immunity with aging and the significance of this observation on immunization strategies.

Current hepatitis B vaccination recommendations used in military immunization clinics follow the package insert guidelines of dosing at 0, 1 and 6 months. It is interesting to note that an extensive literature suggests that dosing at 12 months (or longer) after initial priming doses (2 or 3) is critical for optimizing the immune response (achieving higher titers) and thereby establishing a longer duration of immunity.[5,15,17] Jilg demonstrated that the highest geometric mean levels of HBsAb (> 500 mIU/ml) were seen in the schedules with the last dose at 12 months rather than 6 months (0, 1 and 12 months = 1061 mIU/ml, or 0, 1, 2 and 12 months = 1629 mIU/ml, versus the traditional 0, 1, and 6 months = 280 mIU/ml).[5] This effect was independent of dose since the highest levels of response occurred in the 2 mcg dosing rather than 20 mcg if the last dose was at least 1 year from the first. This observation of the importance of schedule rather than dose and/or route was supported by other investigators including Trivello.[17] Those subjects having received a fourth dose at 14 months had 93.9% (n=653) persistence of protective immunity at 6 years follow-up compared to 67.2% (n=302) for the traditional 6 month schedule group. He also observed a trend of decreasing antibody levels with increasing age. A larger study of active duty personnel with monitoring of their

HBsAb over time following modified strategies of immunization schedule would appear to be necessary to clarify the optimum policy and efficiency for the military as a whole.

One of the impediments to wider immunization delivery is the cost of vaccine weighed against issues of cost benefit or efficacy for disease prevention. Our study data does not address these issues per se. However, the second phase of the study did address the question: can a lower dose of vaccine (1/10th the traditional dose) provide comparable effect in terms of generating a serologic response? As described previously in a study on primary hepatitis B vaccination, 2 mcg intradermally can be very effective in stimulating HBsAb responses with no significant difference compared to a comparable group receiving 20 mcg intramuscularly.[18-24] However, questions have been raised regarding the duration of immunity with these reduced doses and this approach remains unofficial for certain groups of patients with thimerosal hypersensitivity (contraindication to IM injection) and, most recently, patients with renal failure.[25, 26] It is noteworthy that Wistrom once again reported that the duration of protective immunity was linked to the post immunization peak level of antibody and that those individuals with levels above 100 mIU/ml, regardless of route or dose of immunization, demonstrated long term immunity (>90% at 6 years follow-up).[22]

Oliveira's recent report investigating vaccine strategies in health care workers missed the critical importance of timing of vaccine doses (independent of dose or route). He compared combinations of ID and IM dosing but with a fixed schedule, timing the last dose at the less than optimum time of 6 months from the first.[26] Validation of long term efficacy of ID approaches needs to be addressed by larger studies with post vaccine quantitative HBsAb levels and timing of a dose of last vaccine dose at 1 year rather than 6 months. The military

population is ideal for such studies and potentially for long term follow-up of vaccine administration efficacy over time.

In our study, there was a trend toward higher HBsAb levels in those individuals who had received the booster vaccine dose by the intradermal route, however this did not achieve statistical significance and might merit additional study to expand these data. However, since the ID booster response was comparable to the IM dose, our data does support the cost saving procedure of providing 2 mcg ID dosing after a primary series. Since the ID strategy of booster vaccination is effective, a policy of immunizing previously vaccinated service members would be significantly cheaper since the biggest cost of the procedure is the vaccine material itself. However, the methodology of ID injection does require trained personnel to avoid subcutaneous injection where responses can be reduced.[ref - 27??] Additional study may be merited to address the issue of 2 to 5 mcgs of hepatitis B vaccine IM as a booster strategy compared to 2 mcg ID.

For military and travel purposes, new approaches to hepatitis B vaccination may be preferable and merit further study. Marchou and his group reported that 20 mcg IM at 0, 10 and 21 days achieved seroprotection 1 month later in 70% of subjects with booster dosing at 1 year achieving over 95% seroprotection.[28,29] Of note, 93% of vaccine recipients on this accelerated schedule had protective levels before the booster dose at 1 year and 91% were protected at 3 months after the accelerated schedule.

Although there is no civilian official policy regarding serologic follow-up of healthy hepatitis B vaccine recipients, it is noteworthy that recent short reports have again raised the concern regarding hepatitis B infection in perviously vaccinated individuals. Ballinger and Clark report a severe case of acute hepatitis B infection 2 years after the patient had received

the full HB vaccination.[29] A response letter from McIntyre[30] calls for a "powerful cost-benefit analysis" comparing one of two options (with the current no action as unacceptable): regular interval follow-up blood tests versus a single blood test one or more months after the primary series (with a quantitative HBsAb) to plan the need for booster immunization over the subsequent 5 to 10 years. Additional case reports of acute replicative hepatitis B infections after successful vaccination[32] raise concerns about adequacy of HBsAb levels years after primary immunization although a majority of these cases had other chronic illness or immunosuppression as a complicating factor for loss of protective immunity. To what degree this information extrapolates to the healthy population remains a controversy. The additional complicating factor of mutant strains of hepatitis B leaves the playing field unclear at best.[33-35] If the goal in active duty personnel at increased risk is over 95% protective immunity, then the data from our study in the context of the world literature suggests that a change in policy is indicated; i.e., quantitative HBsAb should be measured at some point following the initial vaccine series and booster vaccination strategies should be instituted based on level of HBsAb achieved initially.

Pneumococcal Specific Immunity

Clinical experience in the 1980s demonstrated that *Streptococcus pneumoniae* remains the most frequent microbial cause of community-acquired pneumonia in patients admitted to the hospitals, accounting for approximately 30 to 50% of all cases.[36,37] It is estimated that in 1990 alone there were at least 315,000 to 526,000 hospital discharges for pneumococcal pneumonia (first listed diagnosis) and perhaps as many as 480,000 to 800,000 of all discharged (all-listed diagnoses).[37] The case fatality rate generally accepted is around 5% with deaths in 1990 estimated at 15,750 to 26,500.

The rising concern about antimicrobial resistance in isolates of *S. pneumoniae* coupled with the death of the young and relatively healthy inventor of the muppets, Jim Henderson, has raised public awareness of the threat of these bacteria to life and health.[38-42] During the period 1989 to 1991, 40% of isolates from community-acquired infection and 95% of those acquired in hospital were resistant to penicillin.[38,39] In the US, about 4 to 5 % of isolates have been found as penicillin-resistant but in areas such as Alaska it is as high as 25%.[40-42] Serotypes 6, 14, 19 and 23 have accounted for a majority of the resistant isolates in several countries.[41] These serotypes elicit poor immune responses in young children, further contributing to their virulence.

The pneumococcal vaccine currently available in the U.S. since 1983 contains 23 of the 83 pneumococcal serotypes that cause invasive pneumococcal infections (i.e., isolates from blood, cerebrospinal fluid, other extrapulmonary sites). This vaccine containing serotypes reflect 85 to 90% or more of the types responsible for invasive pneumococcal infections in developed countries. Fedson and Musher (new edition) summarize the current experience regarding immunity to pneumococcal pathogenic serotypes: "most normal subjects lack anticapsular polysaccharide antibody to most pneumococcal serotypes; antibody to a majority of capsular polysaccharide antigens appears after pneumococcal vaccine is administered to healthy young adults."[36]

Our study detailing the prevalence of pneumococcal serotype specific IgG in the serum of a population of healthy active duty personnel (see **Table 6** and **7**) demonstrates significant population vulnerability to pathogenic *S. pneumoniae*, particularly serotypes 6A/B, 7F, 8, 9N and 14 with greater than 20% of the population studied, age and gender independent, lacking protective immunity by the strictest of criteria (< 200 ng Ab N/ml). Using the more widely

accepted level of 250 to 300 ng Ab N/ml, additional serotypes to demonstrate low level protective antibody (detailed data not shown) include the following: PPS-1, 3, 4, 7, 18, 19 and 23, with a trend of lesser antibody levels in younger men (< 34 years) and older women (> 44 years). However, statistical significance was only achieved by age difference in types 9, 12 and 18, and by gender, for type 9 and 18. Overall, these data confirm the observations of Musher[8] and his coworkers who detailed the prevalence of lack of protective immunity in a larger population of military recruits who have been described as a group at risk for pneumococcal infection.[8] Mushner also suggested that subsets of healthy younger adults should be considered for vaccination, particularly if at occupational risk due to cramped quarters and high stress environments.

The high level of non-protective levels of pneumococcal specific antibodies, particularly for serotypes 6A, 7F, 8, 9N, and 14, was surprising since serotypes 6, 7, 9, 14, 18 and 23 account for the large majority of disease isolates in the pediatric population and one would expect higher levels of natural immunity, particularly in young adults.[43] As previously cited, these isolates are poor immunogens in young children (< 2 years of age) so natural infection may not provide long term protective immunologic memory. It is noteworthy that serotypes 6 and 14 are also among the leading players in the penicillin-resistant serotype arena.

Opsonin-dependent phagocytosis is believed to be the major defense mechanism against *S. pneumoniae*. In addition to type specific antibody, most persons produce antibody to cell wall polysaccharide (CWPS) which may be detected in antibody assays due to contamination of capsular polysaccharide antigens. CWPS specific antibodies may play some role in protective immunity but removal from immune sera through an adsorption step has no effect on

opsonization efficiency.[44] Our commercial laboratory assay has been specifically designed to avoid measurement of CWPS antibody by including a specific adsorption step.

A 23 valent pneumococcal vaccine is available for protective immunization of high risk individuals but has not been used in active duty personnel on a routine basis even in settings of occupationally increased risk for infection. An impression that younger healthy populations have adequate natural immunity without immunization is not supported by our study nor reports within the world literature. In fact, for certain serotypes (e.g., 6, 8, 9 and 14), more than 20% of our health study population lacked protective levels of antibody by the strictest criteria of </= 200 ng Ab N/ml of IgG. In the face of a growing public health concern about morbid and/or mortal infections with certain serotypes of *S. pneumoniae*, this avoidance of immunizing younger populations does not appear justified. In fact, military populations may be at even greater occupational risk and this might be reduced by earlier delivery of the vaccine to health care workers, troups anticipated to share close quarters with high stress situations, and other populations with increased infection rates. As newer pneumococcal vaccines become available targeting select high risk serotypes, studies in military populations such as the ranger school might be very fruitful for the future.

Conclusions

1. Hepatitis B vaccine delivery in the absence of an immunization database and improved tracking of immunity status remains short of the over 95% delivery goal. Strategies to improve the adequacy of immunization include the expedited development of a networked, triservice immunization database. Measurement of serology once following completion of a primary series may be justified in select high risk personnel such as health care workers. A serologic level of 500 mIU/ml or greater correlates best with long term (>5 years) persistence of immunity. Targeted boostering at 1 or more years following the primary series also appears justified although further data to clarify the approach in the military setting are indicated.
2. In subjects with a history of a primary vaccine series and low levels of serologic Hepatitis B surface antibody, protective immunity for hepatitis B can be successfully achieved by booster vaccine dosing with 2 mcg dose of a recombinant vaccine by the intradermal route. However, long term studies are needed to determine the duration of the booster response.
3. There is no significant difference by gender in the prevalence of non-protective HBsAb levels and response to booster vaccination by either a low dose intradermal (2 mcg) or standard dose by the intramuscular route. There is a significant trend toward higher antibody responses in women compared to men when given a booster vaccine dose, independent of route, dose or pre-vaccination titer.
4. The newly described accelerated schedule of hepatitis B vaccine administration (0, 10 and 21 days followed by a booster at 1 year) should be studied in the military setting where readiness and need to travel on short notice make the current hepatitis B primary series

cumbersome and frequently less efficacious. Additional study of such an accelerated vaccine schedule with comparison of low dose ID versus IM dosing could improve the military's vaccine delivery and could decrease overall costs.

5. Poor protective antibody levels to pathogenic pneumococcal serotypes exists in younger active duty populations, independent of gender. This observation suggests that immunizing high risk personnel may be justified to reduce morbidity and mortality from pneumococcal disease. Although beyond the time and budgeting constraints of this study, evaluating the use of pneumococcal vaccine in active duty personnel should merit future funding in order to develop a more progressive preventive medicine strategy for the military women and men at greatest risk for pneumococcal disease.
6. A multidisciplinary committee (clinical immunology, infectious disease, pediatrics, preventive medicine and occupational health, etc.) should be established as an ongoing task force responsible for surveillance of the world literature as well as in-house reports of vaccine use issues. Such a task force could prioritize militarily relevant immunization issues for research funding and could anticipate the formulation of changes in immunization strategies that would be advantageous to the military without awaiting the civilian guideline publications that do not necessarily have the same concerns. Such a task force could also be available in times of deployment for expedited review of unique readiness issues including use of experimental vaccines when indicated.

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Table 1

Study Population Enrolled by Gender & Age

Mean Age in Years by Age Groups:	**Total Group** (n=)	**Females** (n=)	**Males** (n=)
All Ages (18-55 yrs)	**33.6** (n = 211)	**35.1** (n = 109)	**32** (n = 102)
GRP 1 (18-34 yrs)	**26.8** (n = 120)	**27.4** (n = 55)	**26.3** (n = 65)
GRP 2 (35-44 yrs)	**40** (n = 61)	**40.2** (n = 37)	**39.7** (n = 24)
GRP 3 (45-55 yrs)	**48** (n = 30)	**48.5** (n = 17)	**47.3** (n = 13)

Table 2:

Hepatitis B Surface Antibody Levels by Gender and Age Category(HBsAb):
Geom Means (SEM) and Range in mIU/ml with Percent of Subjects in Each Group < 20 mIU/ml

Hep B Vaccine Booster Responses	Total	Female	Male
All Study Subjects Enrolled	**211**	**109**	**102**
Geom Mean HBsAb in mIU/ml (SEM): Range:	**127** (14.0) 1 - 271,400	**117** (14.14) 1 - 271,400	**141** (13.98) 1 - 54,400
• Participants with HBsAb < 20 mIU/ml	**46** (21.8%)	**23** (21%)	**23** (22.5%)
• Number with less than 3 shot series	**30**	**19**	**11**
Study Subjects with Primary Series:	**n = 181**	**n = 90**	**n = 91**
Geom Mean HBsAb in mIU/ml (SEM): Range:	**144** (12.52) 1 - 54,400	**135** (11.23) 1 - 36,140	**154** (14.04) 1 - 54,400
• Participants with HBsAb < 20 mIU/ml	**34** (18.7%)	**16** (17.8%)	**18** (19.8%)

Table 3:

Prevalence of Protective HBsAb Levels in High Risk Active Duty Men and Women by Age Group: Documented History of 3 or More Doses of Hepatitis B Vaccine Prior to Entry into the Study

Hep B Vaccine Booster Responses	Total	Female	Male
Study Subjects with Primary Series:	**n = 181**	**n = 90**	**n = 91**
GRP 1: (18 - 34 yrs)	**n = 100**	**n = 42**	**n = 58**
• **HBsAb** in mIU/ml: Geom Mean (SEM) Range:	**237** (12.64) 1 - 54,400	**201** (13.63) 1 - 36,140	**264** (12.36) 1 - 54-400
• Subjects with **HBsAb** < 20 mIU/ml (%)	**14** (14%)	**7** (16.7%)	**7** (12.1%)
GRP 2: (35 - 44 yrs)	**n = 52**	**n = 31**	**n = 21**
• **HBsAb** in mIU/ml: Geom Mean (SEM) Range:	**78** (10.75) $^{p<0.01}$ 1 - 3521	**91** (10.86) 1 - 3521	**55** (13.32) $^{p=0.02}$ 1 - 2030
• Subjects with HBsAb < 20 mIU/ml (%)	**9** (17.3%)	**7** (22.6%)	**8** (38.1%)
GRP 3: (45- 55 yrs)	**n = 29**	**n = 17**	**n = 12**
• **HBsAb** in mIU/ml: Geom Mean (SEM) Range:	**90** (9.79) $^{p=0.05}$ 1 - 4950	**102** (6.66) 1 - 3877	**69** (16.69) 1 - 4950
• Subjects with **HBsAb** < 20 mIU/ml (%)	**6** (21%)	**2** (11.8%)	**4** (33.3%)

Table 4

Immune Response to Booster Immunization with a Recombinant Hepatitis B Vaccine using Two Strategies: 2 mcg Intradermally Versus 20 mcg by the Traditional Intramuscular Route

Hep B Vaccine Booster Strategies:	**Total**	**Female**	**Male**
Intramuscular Route (20 mcg):	**n = 17**	**n = 11**	**n = 6**
IM: Geom Mean HBsAb mIU/ml (SEM): • **Pre-Vaccine** (Range):	**4.1** (4.56) (1 - 43)	**5.6** (2.43) (1 - 43)	**2.4** (3.90) (1 - 17)
• **Post-Vaccine** (Range):	**766** (19.8) (1 - 34,360)	**2175** (13.9)$^{P<0.05}$ (13 - 34,360)	**113** (16.53) (1 - 1808)
• Number of Vaccine Non-responders (%)	**3** (17.6%)	**1**	**2**
Intradermal Route (2 mcg):	**n = 18**	**n = 11**	**n = 7**
ID: Geom Mean HBsAb mIU/ml (SEM): • **Pre-Vaccine** (Range)	**5.2** (3.76) (1 - 44)	**5.6** (4.23) (1 - 44)	**4.7** (3.38) (1 - 18)
• **Post-Vaccine** (Range)	**1150** (23.1) (1 - 31,840)	**3393** (6.41)$^{P<0.01}$ (125 - 31,840)	**210** (59.4) (1 - 11,103)
• Number of Vaccine Non-responders (%)	**3** (16.7%)	**0**	**3**

Table 5:

HBsAb Response to Booster Immunization with a Recombinant Hepatitis B Vaccine:
Comparison of Gender Responses Independent of Route (IM vs ID) and Dose (20 mcg vs 2 mcg)

Hep B Vaccine Booster Responses	Total	Female	Male
Intramuscular Route (20 mcg): **Intradermal Route (2 mcg):**	17 18	11 11	6 7
TOTAL: IM + ID Routes:	**35**	**22**	**13**
Geom Mean HBsAb (mIU/ml): • **Pre-Vaccine** (Range):	**4.7** (4.08) (1 - 44)	**5.6** (4.36) (1 - 44)	**3.5** (3.60) (1 - 18)
• **Post-Vaccine** (Range):	**944** (20.66) (1 - 34,360)	**2716** (9.35) $^{p=0.01}$ (13 - 34,360)	**158** (30.7) (1 - 11,103)
• Number of Vaccine Non-responders	**6** (17%)	**1**	**5**

Table 6

Pneumococcal Polysaccharide Serotype Specific IgG (ng Ab N/ml) by Gender & by Age Grouping

Geometric Mean Pneumococcal Polysaccharide Capsular Specific IgG with SEM (Range)						
Pneumococcal (PPS) Serotype #:	**Total** **n = 211/210***	**Female** 18-55 yrs **n = 109**	**Male** 18-55 yrs **n = 102/101***	**Grp 1** 18-34 yrs **n = 121**	**Grp 2** 35-44 yrs **n = 60**	**Grp 3** 45-55 yrs **n = 30/29***
PPS-1 SEM (Range)	525 2.33 (50-16080)	562 2.27 (70-16080)	**487** 2.39 (50-2900)	**498** 2.42 (50-16080)	603 2.11 (70-2901)	**491** 2.42 (110-2901)
PPS-3	877 3.01 (60-33884)	979 3.17 (60-33520)	779 2.81 (90-7700)	846 3.29 (90-33520)	1039 2.45 (150-8110)	719 2.97 (60-5121)
PPS-4	878 2.84 (71-22909)	956 2.82 (80-13040)	800 2.84 (70-22961)	819 2.78 (70-13040)	1100 2.86 (120-22961)	731 2.90 (80-3100)
PPS-6	**325** 2.13 (30-4266)	**338** 2.12 (50-4287)	**311** 2.15 (30-3780)	**313** 2.20 (30-4287)	**354** 1.98 (50-3780)	**314** 2.19 (80-2310)
PPS-7	**389** 2.13 (30-4266)	**425** 2.22 (30-2940)	**355** 2.03 (60-1490)	**365** 2.08 (60-2940)	**456** 1.90 (60-1370)	**370** 2.77 (30-1710)
PPS-8	**166** 2.00 (30-1905)	**173** 1.96 (40-1900)	**158** 2.04 (30-1590)	**151** 1.98 (30-1650)	**183** 1.71 (50-560)	**199** 2.53 (50-1900)
PPS-9♣♦	**291** 2.02 (50-3162)	**320** 2.02 (70-1480)	**262** 1.99 (50-3201)	**253** 1.97 (50-1760)	**346** 1.94 (80-3201)	**358** 2.13 (80-1480)
PPS-12♣	906 2.73 (71-17378)	975 2.65 (130-17298)	837 2.81 (70-16319)	822 2.74 (70-17398)	1208 2.80 (130-16319)	750 2.27 (170-2040)
PPS-14	**399** 2.57 (40-3981)	**437** 2.79 (40-3952)	**362** 2.32 (50-1290)	**383** (50-3952)	**438** (60-1972)	**392** (40-1290)
PPS-18♣♦	616 2.42 (40-3981)	725 2.45 (120-3901)	517 2.32 (60-3901)	526 2.33 (60-3260)	828 2.36 (140-3901)	648 2.60 (130-3901)
PPS-19	740 2.26 (110-18198)	780 2.31 (110-5301)	701 2.20 (150-18281)	697 2.33 (130-18281)	864 2.01 (110-5170)	696 2.42 (120-3960)
PPS-23	640 2.39 (60-8128)	689 2.52 (60-8220)	591 2.23 (110-5410)	591 2.45 (60-8220)	737 2.56 (150-5410)	663 2.36 (160-2920)

* N is one less for serotypes 1,4,6,8,12,18,19 & 23;

♦ = significant difference between men & women (p = .0346 for serotype 9 and p = .0074 for serotype 18);

♣ = significant difference between age groups (p = .0027 for serotype 9; p = .0419 for serotype 12; and p = .0105 for serotype 18)

Table 7

Percent (%) of Subjects with Non-Protective (≤ 200 ng Ab N/mL) Levels of Pneumococcal Antibodies

Pneumococcal Serotype #: % </= 200 ng Ab N/ml	Total n = 211	Female 18-55 yrs n = 109	Male 18-55 yrs n = 102*	Grp 1 18-34 yrs n = 121	Grp 2 35-44 yrs n = 60	Grp 3 45-55 yrs n = 30/29*
PPS-1	11	9	14	12	7	17
PPS-3	8	10	7	10	3	13
PPS-4	8	7	8	9	2	14
PPS-6	28	24	32	29	23	31
PPS-7 ♦	18	13	24	21	12	20
PPS-8	61	59	63	66	56	52
PPS-9 ♦ ♣	31	25	39	39	20	23
PPS-12	6	5	7	8	2	3
PPS-14	26	26	28	29	20	30
PPS-18	10	10	11	12	7	14
PPS-19	6	6	5	7	2	10
PPS-23	10	9	10	11	8	7

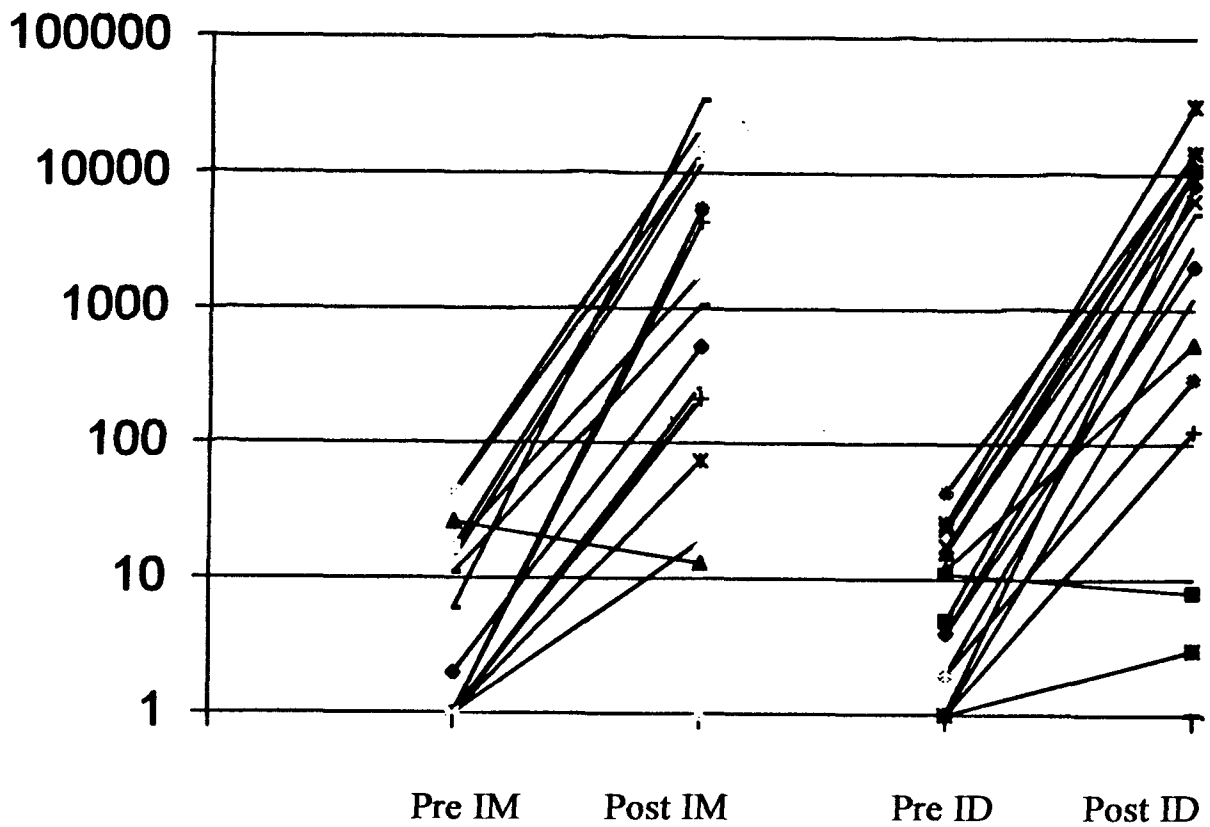
* N is one less for serotypes 1,4,6,8,12,18,19 & 23

♦ = significant difference between men and women: for serotype 7 p = .0440; for serotype 9, p = .0355

♣ = significant difference between age groups: p = .0222

FIGURE 1

Hepatitis B Ab Levels
Pre/Post Boost
Intramuscular vs. Intradermal



GEOM MEAN (mIU/mL):

Pre IM = 4
Post IM = 766
(n = 17)

Pre ID = 5
Post ID = 1150
(n = 18)

Personnel Receiving Pay from
Military Interdepartmental Purchase Request 95MM5537

Three different protocol nurses were hired (consecutively):

Dr. Stan Silverman	November 1, 1994 - April 30, 1995
Judy Brooks	May 15, 1995 - September 15, 1995
Gail Bergan	September 16, 1995 - December 31, 1995

From JACI 97 (1) pt. 3, p. 399 (#867), January 1996:

867 **Delayed Type Hypersensitivity (DTH) Skin Testing (SKT) for the Evaluation of Cellular Immunity: Normal Responses for Adult Men and Women.** A. Heiser, MD, R DeGuzman, MD, J Brooks, RN, N Veltri, RN, V Carregal, MD, LS Smith, MD, GB Carpenter MD, RJM Engler MD. Walter Reed Army Medical Center, Washington, DC.

The assessment of functional cellular immunity has long incorporated DTH skin testing with "common recall" antigens such as tetanus (Tet), candida (Can), mumps (Mum) and trichophyton. The absence of measurable induration (≥ 5 mm) 48 hours after intradermal injection of 0.1 ml of antigen, or "anergy", remains a useful screening test in the evaluation of both primary and secondary cellular immunodeficiency. There is limited normal population data regarding the number of tests required to define a "normal" response. We evaluated DTH SKT responses in 106 healthy subjects (ages 18-55) using Tet and Can antigens; 83 also had a mumps DTH SKT placed. The frequency (%) of positive reactions (≥ 5 mm diameter) are summarized below for all subjects, and subgroups by gender: females (F) and males (M):

	n =	% Pos	% F (n =)	% M (n =)
Tet	106	91.5	87.9 (58)	95.8 (48)
Can	106	93.4	77.5 (58) $p<.02$	91.7 (48)
Mum	83	100	100 (46)	100 (37)

It is noteworthy that women had significantly less frequent DTH reactions to Candida than men. Since candida vaginitis is a common mucocutaneous infection in women, this was an unexpected finding.

Conclusion: The absence of anergy can be defined using only three (3) DTH skin test antigens (Tet, Can & Mum). Mumps antigen (at 40 U/ml) resulted in 100% reaction rate in our population. We speculate that the increased use of MMR vaccines may explain this observation.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 9 Mar 98

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for the following contracts. Request the limited distribution statement for these contracts be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

Contract Number	Accession Document Number
DAMD17-94-J-4407	ADB224557
DAMD17-95-1-5048	ADB230013
DAMD17-95-C-5006	ADB219041
95MM5508	ADB227588
95MM5522	ADB229897
95MM5537	ADB227721
95MM5596	ADB229924
96MM6652	ADB220033
96MM6653	ADB221466
96MM6654	ADB222409

2. Point of contact for this request is Ms. Betty Nelson at DSN 343-7328 or email: betty_nelson@ftdetrck-ccmail.army.mil.

FOR THE COMMANDER:

Phyllis Rinehart

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Completed
2-8-2000
BNC